Applied Soil Ecology 101 (2016) 152–164

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

The activity of mycorrhizal symbiosis in suppressing Verticillium wilt in susceptible and tolerant strawberry (*Fragaria* x *ananassa* Duch.) genotypes

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ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 25 January 2016
Accepted 27 January 2016
Available online 12 February 2016

Keywords:
Verticillium dahliae
Disease tolerance
Water balance
Chlorophyll fluorescence
Strawberry
Arbuscular mycorrhiza

ABSTRACT

Verticillium dahliae is a soil borne plant pathogen that causes Verticilium wilt disease in strawberry plants. Arbuscular mycorrhizal (AMF) symbiosis plays a major role in plant's ability to withstand numerous abiotic and biotic stresses, including pathogenic fungi infections. This study was conducted to test the effect of mycorrhization with arbuscular mycorrhizal fungi on reaction of strawberry plants susceptible (cv. 'Elsanta') and tolerant/resistant (cv. 'Senga Sengana' and K40 clone) to *V. dahliae* infection. Response of mycorrhized plants to pathogen infection was determined visually as disease severity and by measuring plant water status (leaf water potential, stomatal conductance, transpiration rate and relative water content) and photochemical activity, using chlorophyll *a* fluorescence method. AMF significantly suppressed disease development in highly susceptible 'Elsanta' and moderately in 'Senga Sengana' and somaclone K40. Inhibition of disease development in 'Elsanta' plants was accompanied by increased stomatal conductance, transpiration rate and, as result, leaf water potential. Photochemical activity, measured as chlorophyll *a* fluorescence parameters, decreased with disease development only in 'Elsanta' plants and AMF significantly counteracted this effect. AMF did not have an effect on photochemical activity in the cultivars tolerant/resistant to *V. dahliae*.

1. Introduction

Verticillium dahliae Kleb. (Hyphomycetes, Ascomycota, Fungi) is a soil borne plant pathogen that causes wilt disease in many agronomically important species, among them in strawberry (*Fragaria* x *ananassa* Duch.). The fungus' hyphae enter the roots and then develop inside vascular tissues disturbing water uptake. As a result, plant water status and photosynthetic activity progressively decrease leading to plant decline (Bowden and Rouse, 1991; Fradin and Thomma, 2006; Garmendia et al., 2005; Hernandez-Sebastia et al., 1999; Lorenzini et al., 1997; Pascual et al., 2010).

There are marked differences in susceptibility to *V. dahliae* among strawberry cultivars. Among dessert cultivars, 'Elsanta' is known as highly sensitive to *V. dahliae* and severe losses, up to 80% of yield, can occur during season favorable for disease development. 'Senga Sengana' is considered as highly tolerant (Grant et al., 2010; Shaw et al., 2010; Zebrowska, 2011). The K40 clone, selected *in vitro* as a somaclone of 'Elsanta', is significantly more tolerant/resistant to infection with *V. dahliae* than both original 'Elsanta' and 'Senga Sengana' (Sowik et al., 2001, 2004).

Arbuscular mycorrhizal (AMF) symbiosis plays a major role in ecosystems, facilitating nutrients and water cycling and also promoting plant's ability to withstand numerous abiotic and biotic stresses (Auge et al., 2015; Borowicz, 2010; Garmendia et al., 2004, 2005; Harrier and Watson, 2004; Kapulnik et al., 2010; Kobra et al., 2011; Li et al., 2014; Zhu et al., 2010). Mycorrhizal symbiosis plays also a key role in soilless production, including *in vitro* techniques (Bharadwaj et al., 2012; Borkowska, 2002; Borkowska et al., 2008; Gianinazzi et al., 1990; Taylor and Harrier, 2001). The plants produced by this method are free from any microorganisms, also those beneficial. Thus, mycorrhization procedure is being included to protocols of micropropagation in order to produce certified stock of strawberries and facilitate better plant adaptation to *ex vitro* condition and their further performance in the field (Borkowska, 2001, 2002; Martins, 2008).

It is well documented that photosynthesis is very sensitive to water deficit, with photosystem II (PSII) being specifically affected due to disruption of electron transport. Since both mycorrhizal symbiosis and infection with *V. dahliae* are having an impact on plant's water status, chlorophyll (Ch) fluorescence has been used as

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http://dx.doi.org/10.1016/j.apsoil.2016.01.021
0929-1393/

a powerful and reliable, non-invasive method for assessing the changes in the functioning of PSII of infected plants by both pathogen (*V. dahliae*) and symbiont (AMF) and accompanied drought stress (Auge et al., 2015; Borkowska, 2002; Borkowska et al., 2008; Faraloni et al., 2011; Pascual et al., 2010; Proctor and Smirnoff, 2000; Sadras et al., 2000; Vodnik and Gogala, 1994; Wright et al., 2009; Zhu et al., 2010).

Controlled mycorrhization has been introduced to commercial horticultural practice. Although the beneficial role of mycorrhizal symbiosis has been frequently observed, reported have been also cases where mycorrhizal inoculation had no effect or even caused decrease in plant productivity (Jifon et al., 2002; Schroeder and Janos, 2004). Thus, question put by Correa et al. (2006): "Are mycorrhiza always beneficial?" is still actual and legitimizes undertaking our investigations.

This study was addressed the following problems:

- How effective is mycorrhization in suppressing Verticillium wilt development in strawberry genotypes differing in susceptibility to the disease.
- Are these responses related to water status and phytochemical activity.

2. Material and methods

2.1. Plant material and fungal inocula

The experiment was conducted in the greenhouse on micropropagated strawberry (*Fragaria x ananassa* Duch.) genotypes: cv. 'Elsanta' (susceptible), cv. 'Senga Sengana' and K40 somaclone (highly tolerant).

Micropropagation was done according to Boxus (1974) method, modified in our laboratory (Borkowska, 2001). After eighth subcultures, microshoots were rooted *ex vitro* in presence of AMF added to substrate. Mycorrhizal inoculum was provided by Agrauxine (France), previously BIORIZE, and consisted of *Glomus intraradices* (according to Li et al. (2014), recently renamed *Rhizophagus intraradices*) or *Funneliformis mosseae* and *Glomus* species.

During rooting and acclimatization the plants were maintained in the growth chamber at 21–23 °C under irradiance 75 μmol m^{-2} s^{-1} provided by warm white fluorescence tubes (Philips) and 16/8 h photoperiod. Then the plants were transplanted into the pots filled with a mixture (1:5, v/v) of sand and soil (Blumenerde, Athens) and transferred to greenhouse. In the greenhouse, additional lighting was provided to maintain 16/8 h photoperiod. Nutrient management of plants was provided according to standard practices for strawberries, however with fertilizer containing low P, produced for purpose of our experiments by polish firm Intermag. The plants were watered as needed.

Three weeks after mycorrhization half of the plants were infected with *V. dahliae*. The pathogen was isolated from the heavily infested experimental field, which is used for testing strawberry plants susceptibility to Verticillium wilt. The isolated fungi were cultured for 14 days on Malt Extract Agar medium (Sigma–Aldrich) and then transferred to liquid malt extract medium. After 21 days the fungal mycelium was homogenized together with the growth medium and inoculated on sterile substrate containing sand, cornmeal and water (10:1:2, v/v/v). After 3 weeks of incubation at 21 °C, when the whole substrate was overgrown with the fungus, it has been used for inoculating soil substrate in the pots with tested plants. In order to facilitate faster infection, the plant's roots were slightly injured with a sharp stick.

Part of the plants was left free of both AMF and pathogen and were treated as control. The roots of control plants were also injured but the soil substrate was treated with sterile mixture of sand and cornmeal instead of the pathogen.

2.2. Treatments

In our studies, there were 4 types of treatment of strawberry plants: (1) inoculation with *V. dahliae* (Verticillium); (2) mycorrhization and three weeks later inoculation with *V. dahliae* (AMF + Verticillium); (3) mycorrhization alone (AMF); 4) plants not mycorrhized and not inoculated with *V. dahliae* (control).

2.3. Measurements

2.3.1. Determination the effectiveness of inoculation with AMF

For determining the extent of AMF colonization, samples of apical parts of roots were harvested from five plants and stained, according to procedure proposed by Phillips and Hayman (1970). Presence of AMF was assessed using microscope.

2.3.2. Disease progress evaluation

Disease incidence and severity were assessed with visible symptoms along the growth period. Disease severity was rated on a scale 0–5, where: 0—healthy shoots, all leaves green; 1—single leaves yellowish-brown; 2—<25% leaves yellowish-brown; 3—26–50% leaves yellowish-brown; 4—51–75% leaves yellowish-brown; 5—76–100% leaves yellowish-brown, the plants dead (Sowik et al., 2001).

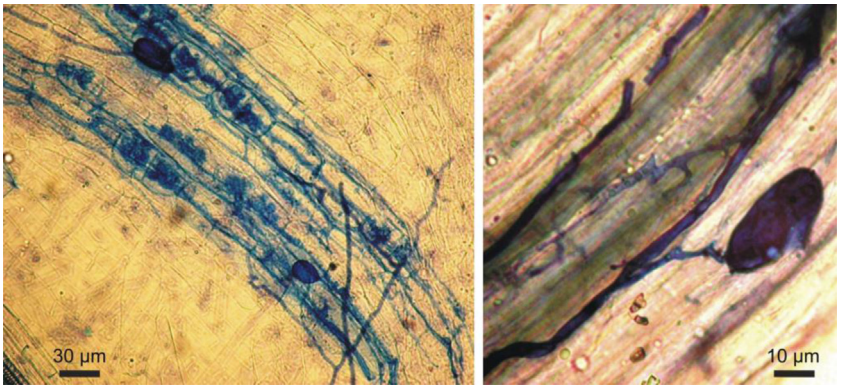

Fig. 1. Sample photographs showing arbuscular mycorrhiza in strawberry roots (vesicles, arbuscules and intraradical hyphae).